BIOLOGICAL ASPECTS OF THIOLS: PROTEIN STRUCTURE, ANTIOXIDANT DEFENSE, CELL SIGNALING AND REDOX STATES

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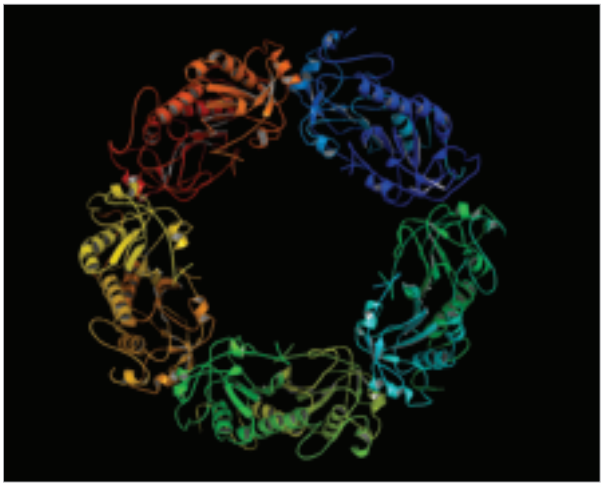

Figure 1. Tsa1 decameric crystal structure. Crystal of yeast Tsa1C47S was obtained by hanging-drop vapor diffusion in the presence of sodium citrate pH 4.2, 10 mM sodium chloride and 10% (w/v) PEG 3000 with 100 mM sodium fluoride as an additive. Each monomer is represented here by a different color. Figure was generated by the Pymol software (www.pymol.org). These results were published in Oliveira et al. *2007.*

The classical concept of oxidative stress is "a disturbance in the pro-oxidant-antioxidant balance in favor of the former". However, the accumulation of data has lead some authors to suggest that a more useful definition could be a "disruption of redox signaling and control". The tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) plays a central role in the redox homeostasis, but there are also other thiols that participate in redox signaling. These thiols, which participate in redox pairs (RSH/RSSR), are enzymes that contain reactive cysteines (such as thioredoxins, glutaredoxins and peroxiredoxins), and are widely distributed. In contrast, most cysteine residues, both free and in proteins, possess low reactivity to undergo redox transitions. Appropriate protein folding in oxidoreductases generates environments in which cysteine residues are reactive. In this project, we propose to characterize both structurally and functionally several thiol systems, especially those derived from the model organism *Saccharomyces cerevisiae*. We have already elucidated the structures of several proteins that compose these systems and now we intend: (1) to elucidate novel structures; (2) continue to make functional-structural correlations and (3) determine the structure of protein complexes. Among these studies, we intend to further investigate a new antioxidant pathway: the reduction of 1-Cys peroxiredoxins by ascorbate (vitamin C). Our studies have changed the "thiol specific antioxidant paradigm" of these thiol-disulfide oxido-reductases and opened the perspective that ascorbate can interfere in the redox states of several thiol systems and, consequently, in redox signaling. We also intend to continue our characterization of antioxidant systems from *Xylella fastidiosa*. Previously, we elucidated the first structure of the organic hydroperoxide resistance protein (Ohr) from *Xylella fastidiosa*. Since Ohr is exclusively present in bacteria, this protein may represent a promising target for drug development. Unique structural and functional features of Ohr lead us to propose Ohr/OsmC as a novel family of antioxidant proteins. Other antioxidant systems from *Xylella fastidiosa* are also being currently analyzed.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We are studying the physiological roles of thiol-proteins by employing multiple approaches such as enzymatic assays, crystallographic studies and microbiological investigations, by using yeast as a model. In this regard, we have purchased a collection of thousand of strains, each one with a different gene deleted. Detailed analyses indicated that although some of these proteins are partially redundant, they do present specific roles. As an example, glutaredoxin 1 and glutaredoxin 2 share a high degree of amino acid sequence identity (64%), but the latter enzyme is fifteen times more active than the former with respect to the monothiol mechanism. Several potential features involved in this phenomenon were postulated through structural analyses and we are in the process of testing them by site-specific mutagenesis. On a more dramatic example, Tsa1 and Tsa2 share 86% of amino acid sequence identity and present significant differences in the pK_a values of their reactive cysteine. We are currently refining a decameric crystallographic structure of Tsa1 (fig. 1), and this might provide us with information on its functional divergence. We suspect that these structural-functional variations may be related to protein-protein interactions, therefore we are also pursuing the elucidation of the structures of protein complexes. Another significant achievement of this project is the finding that ascorbate (vitamin C) can support the peroxidase activity of 1-Cys peroxiredoxins, which represents a change in the thiol-specific antioxidant paradigm. Finally, it is important to mention that our group was responsible for the description of the first structure of the Organic Hydroperoxide Resistance Protein from *Xylella fastidiosa* (fig. 2). The detailed characterization of this enzyme revealed unique features and led us to propose Ohr/OsmC as a novel family of antioxidant proteins. Since Ohr/OsmC proteins are exclusively present in bacteria (most of them pathogenic), Ohr appears to be a promising target for drug therapy, and in this regard, we are in the process of searching for potential inhibitors.

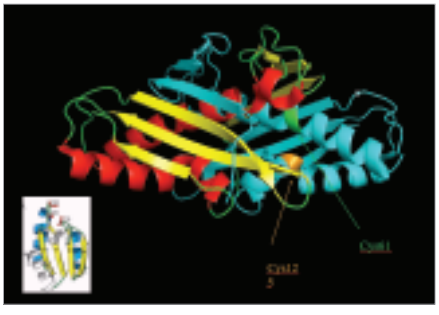

Figure 2. Ohr dimeric crystal structure. Crystal of Xylella fastidiosa *Ohr was obtained by hanging-drop vapor diffusion in the presence of 25% (w/v) PEG 4000 and 0.1 M Tris–HCl buffer (pH 8.7). One monomer is depicted in light blue while the other is in red, yellow and green. Cysteine residues involved in catalysis are shown in green and orange. Figure was generated by the Pymol software (www.pymol.org). These results were published in Oliveira* et al. *2006.*

MAIN PUBLICATIONS

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